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DER #3

Fluvalinate: 6-Month Oral Toxicity Study in Dogs
Zoecon Corporation. 1980. MRID No. 00077025, 92069033, 92069056.
HED Doc. No. 001786, 002256, 004707.

83-1(b)
anemia

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

004702

February 29, 1984

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: MAVRIK; Splenic Weight Changes in Dogs

TO: Albin Kocialski, Head, Acting Section II
Toxicology Branch/HED

FROM: Marion Copley, Toxicologist, DVM
Section II
Toxicology Branch/HED

Caswell #934

After studying the 90 and 180 day dog reviews and the sponsors response to your questions about splenic weight changes, I have made the following observations:

1. Stress will, as the sponsor states, cause splenic contraction, a condition unique to the dog as opposed to rats and of much less importance in man. The stress of the dog, for animals already acclimated to the test conditions, probably would not, by itself, be enough to cause the significant weight changes observed.

2. Other means of stress (i.e., biological) will result in splenic contraction and when combined with conditions described by the sponsor, could be responsible for the splenic weight difference.

3. Ninety-day hematologic tests are suggestive of anemia. This could result in biological stress and by itself could be responsible, at least in part, for the lower spleen weights. It would be interesting to compare the individual animal hematologic parameters with spleen weights.

4. I was not able to determine if the hematologic changes were present at 180 days.

5. Without histologic splenic changes and in view of the 180 day dog study with no splenic changes, I feel that the sponsor is justified in considering the effect to be unrelated to a direct pharmacological or toxicological effect of the test agent.

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RESULTS

180-Day Dog Study

The results and discussion of this review cover the experimental period between days 90 and 180 with the exception of skin lesions (clinical observations) which cover the period 1 thru 180 days. This report is (or should be) found appended to the results and discussion which cover the days 1 thru 90 of the 180-day dog study.

REFERENCES

Subject: Six-Month Subchronic Dog Study. Final Report.

Test Compound: ZR-3210 Technical (Racemic Mixture), Fluvalinate, Mavrik®

Accession Nos: 070097- 98; 99

Testing Facility: Elars Bioresearch Laboratories, Inc., Fort Collins, Colorado 80524

Project No: 1503

Testing Period: December 21, 1979 - April 13, 1980

Report Submitted to Sponsor: June 1980

Purity of Test Material: 93.8% (first shipment); 93.0% (second shipment)

Batch or Lot No: Analysis No. 0979-069 Run 7 (first shipment); Analysis No. 0280092 (second shipment)

Stability: Stable for at least 12 months when stored in sealed glass containers and exposed to artificial light at 25 and 42°C. Stable in preparation and under the conditions of the study.

Clinical Observations: Emesis: The most frequently detected dose-related sign was emesis which occurred frequently in all dogs and almost daily in some dogs receiving 50 mg/kg/day. Accomodation to the test material in terms of a decreased frequency of emesis was not observed. Emesis occurred infrequently in the control group and generally to the same extent in those animals receiving 2 and 5 mg/kg/day. The frequency and quantity of emesis in animals receiving 15 mg/kg/day generally ranged from 2-15 times greater than that seen at the lower dose levels. The frequency and quantity of emesis in dogs receiving 50 mg/kg/day was 5-13 times greater than that seen at 15 mg/kg/day.

Diarrhea: The frequency of diarrhea between the control group and the two lower dose levels was generally comparable. The frequency of diarrhea at a dose level of 15 mg/kg/day was generally 9-20 times greater at this dose level than at the lower dose levels and 2-12 times greater at 50 mg/kg/day than that seen at 15 mg/kg/day.

Dehydration: In the Toxicology Branch review of the 90-day interim kill report to the 180 day dog study, it was noted that dehydration occurred in several dogs receiving the high dose and appeared consistently in 4 females and 1 male. Three of these dogs (all females) were sacrificed at the 90-day mark. The male (MH-103) which had received lactated Ringers Solution as an emergency treatment for one week remained somewhat dehydrated but stable and responsive through out the remainder of the study. The female (WZ-89) did not receive lactated Ringers Solution and did remain somewhat dehydrated (0-5%), but stable and responsive through the 180 day period.

During week 18 of the study, one female (UB-89) from Group IV (15 mg/kg) became severely depressed and dehydrated. The animal's condition deteriorated steadily, with rapid weight loss, anorexia, and the appearance of multiple abscesses. The animal was euthanatized on day 159 of the study.

Skin Lesions: Localized skin lesions persisting for several weeks were reported in all groups receiving the test article (see attached). Although a log-dose response was not evident, a compound related effect appeared to be present. A tabular representation of data for those animals showing at least one lesion at any time period from 1 to 180 days is reproduced below.

Number of Lesions Observed at Any Time Period (180 Days)

Group	Male	Female	Total
I	0	0	0
II	0	1	1
III	2	5	7*
IV	2	3	5
V	4	4	8

* One male had OTITIS prior to testing and was erroneously counted as having a lesion at 90 days. This number is one less than reported at 90 days.

One male and one female each in group IV and V which did not manifest lesions during the 1-90 day period did manifest lesions after 90 days.

Lesions were generally described as an open sore, irritation, or inflammation. Open sores were generally in the neck area or the right hind paw. Irritations were associated with the prepuce and vulva. Inflammation and/or irritation were terms used to describe findings in or on the ears.

Examination of the table shows that the number of lesions appeared to be nearly equally distributed between sexes and that no lesions were recorded for controls.

One female (UB-89) of Group IV had an open sore of the neck 60 days post dosing which lasted for 8 weeks. This animal was treated with nitrofurazone powder. Approximately 75 days later this same animal manifested abscesses of the face, neck, and right front paw. Bacterial culture results were negative for the neck but positive for the face (unidentified species of Staphylococcus). No culture results were taken of the paw. This animal (UB-89, see also dehydration paragraph) was sacrificed at 159 days due to a severe deterioration of its general health.

It was reported that dogs with lesions appeared to suffer from pruritus; they licked, chewed or scratched the affected areas repeatedly. The pruritus appeared to be more severe shortly after dosing, since dogs which were quiesccent in the morning chewed and scratched themselves two to three hours after they were dosed.

Numerous treatments were attempted. The affected areas were wrapped or bandaged, Elizabethan collars were placed on the dogs and finally, if necessary, various topical medications were applied. The application of medication (topical antibiotic) was reserved only for the most refractory cases. Staphyloccocus aureus was identified in 3 dogs and beta-hemolytic streptococcus in 2 dogs. Staphylococcus, unidentified as to species, was also diagnosed in a sixth dog.

Body Weights: Mean body weights for males in the high dose group revealed statistically significant ($p < 0.05$) decreases from week 19 to 26 inclusive when compared to the control group. Values for females were not statistically significantly lower at any time period. The combined male and female body weights were not statistically significantly lower than combined control weights at any time period.

Graphical representation of male body weight data shows parallel increases in body weight and non-significant departures from controls for Groups I thru IV thru termination. Group V, the high dose group, departs from all other groups at about 6 weeks and remains flat for the remainder of the study.

Graphical representation of female body weight data shows parallel and non-significant divergence from controls for Groups III and IV while Group II shows parallelism and is substantially above the control group. Body weights for females of Group V are essentially flat for weeks 1-4. Body weights for weeks 4-26 are parallel but substantially below controls.

The total weight gain for males in the 50 mg/kg/day dose group was statistically significantly lower at the end of 26 weeks when compared to all other groups.

The total body weight gain for females in the 50 mg/kg/day dose group was substantially lower than all other groups but the decrease was not statistically significant.

The combined values for sexes were substantially lower for the 50 mg/kg/day group, but not statistically significant. All other group values were comparable to control values.

Food Consumption: Food consumption was not statistically significantly different from control values for either sex when considered separately or in combination.

Opthalmic Examinations: Terminal opthalmic examinations were performed on all surviving dogs prior to necropsy. The examinations were reportedly carried out on a blind basis and subsequently compared to the 90 day examination. No significant changes from the 90 day examination were observed. No substantial changes were observed in the results from the Schirmer tear test.

Neurologic Examination: Observations reported for animals at 180 days (note here that only three groups were examined for neurologic abnormalities - they were the vehicle control group, the 5.0 mg/kg/day group and the 50.0 mg/kg/day group) revealed the following; 12/12 control animals were reported as normal; 12/12 dogs in the group receiving 5.0 mg/kg/day were reported as normal; and 11/12 dogs in the high dose group showed no abnormalities. Only one animal, a female, which showed hyporeflexia of the right and left rear patella at 90 days continued to show the same sign at 180 days.

Clinical Pathology: Hematology: The Red Blood Cell Count (RBC) for males and females of the high dose group at 180 days, although not statistically significant, was much lower than control values. However, the combined values for males and females for the same dose and time period were statistically significantly ($p < 0.05$) lower when compared to the combined values for the control group. The hematocrit was statistically significantly lower in the high dose group for the combined male and female values at the 180 day reading. However, values for males, females and their combined values were depressed when compared to control values at all time periods (i.e., 120, 150 and 180 days) for the high dose (50 mg/kg/day) group. Hemoglobin values for male and female animals showed successive decreases at 180 days with increased dose. None of the values were however statistically significant. The combined values for males and females showed successive decreases in hemoglobin (Hg) values with an increased dose at 180 days. The dose of 50 mg/kg/day produced a statistically significant decrease. Platelet count was not statistically significantly different at any dose level at any time period. Activated Partial Thromboplastin Time (APTT) and Protime (prothrombin time) values were comparable to control values.

No statistically significant changes were noted for values of mean corpuscular hemoglobin. The mean corpuscular hemoglobin concentration was statistically significantly lower than Groups I, II and III for males in the high dose group at 180 days. No other biologically meaningful changes were observed at any time period. White blood cell count (WBC) and WBC differential count did not appear to show any biologically meaningful differences in the context of compound administration even though some values were statistically significant. These changes occurred randomly. Methemoglobin values taken on day 150 only were not statistically significantly different at any dose level for males or females when considered either separately or together. Total protein was statistically significantly decreased at various time intervals and doses. However, no log-dose response was evident and no biologically

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meaningful decreases with time were observed. The statistically significant decreases appeared to occur in a random manner. Albumin values for males were statistically significantly decreased in the high dose group at 180 days. Values for females were statistically significantly decreased at 120 and 180 days in the high dose group. Values for females were depressed in the high dose group at 150 days when compared to controls. The combined values for males and females, when compared to controls, were statistically significantly lower than controls at all three time periods (i.e., days 120, 150, and 180) in the high dose group. Additionally, combined values in the high dose group were statistically significantly lower when compared to two other dose groups, beside control group, at the 120 and 180 day reading. Globulin values were statistically significantly increased only for the combined values for sexes at 120 days in the high dose group when compared to controls.

The albumin/globulin ratio (A/G ratio) was statistically significantly decreased only for females and the combined values for males and females at 120 days in the high dose group. Values for glucose were not statistically significantly different from control values at any dose level or time period. Values for Blood Urea Nitrogen (BUN) were not statistically significantly different from control values at any dose level or time period. Total bilirubin was comparable for all groups at all time intervals. Alkaline phosphatase values were comparable for all groups at all time intervals. Calcium values at 120 days were statistically significantly decreased in the high (50.0 mg/kg/day) dose group for males and the combined values for males and females. Calcium values were also statistically significantly lower at 180 days for the combined values of the sexes at the high dose. It is also pointed out here that inspection of the data reported for 180 days revealed lower calcium values in the high dose group as apposed to all other groups for both males and females. Values for 150 days appeared comparable.

Values for Na and K were not statistically different between groups at any time period. SGOT and SGPT values for males and females were not statistically significantly different between groups at any time period. Cholesterol values were comparable for treated groups when compared to controls at all time periods. LDH values were comparable between treated groups and control groups. RBC cholinesterase corrected values were not statistically significantly decreased

at any time period for any test group [Note: corrected values were reported for 60, 90 and 180 days. Only absolute values were originally reported for 60 and 90 days and showed values to be statistically significantly lower for males, females and their combined values at 90 days. This reviewer argued that the decreased RBC cholinesterase values at 90 days were probably the result of a decreased hematocrit. The absolute RBC cholinessterase values corrected for a decreased hematocrit reveal no values statistically significantly different from the control group. Corrected values were calculated by dividing the mean control hematocrit by the individual hematocrit and multiplying by the absolute RBC cholinesterase value for each dog]. Serum and brain cholinesterase values were comparable at all doses and time intervals. Values for specific gravity (urine) and ph (urine) were presented for combined sexes and were not statistically significantly different from the control group values at 120, 150 and 180 days. No statistically significant differences were noted among groups for the other parameters listed at 120, 150 and 180 days for urinalysis.

The absolute terminal body weights for male dogs were statistically significantly lower only for the high dose group when compared to the control values.

Absolute organ weights were not significantly different between treated animals and controls. Organ to body weight ratios were statistically significantly greater for liver, kidney and adrenal only for males in the high dose group when compared to any other group. Organ to brain weight ratios were statistically significantly greater for males in the high dose group only for liver. Brain weight ratios for kidneys, liver and adrenals were not statistically significantly increased when compared to controls at dose levels of 15 and 50 mg/kg/day for females.

Pathology: The salient pathological findings are noted below with their respective doses for dogs continued on the experiment after 90 days.

Dose: 5.0 mg/kg/day. Male dog DK 99. Skin. Right hind paw lesion and left leg lesion, hyperkeratosis (moderate severity) inflammation, chronic (moderate severity).

Dose: 15.0 mg/kg/day. Male dog AW 99. Skin paw. Right hind paw, hyperkeratosis (moderate severity), inflammation, chronic (moderate severity). Skin neck, a scabbed sore, inflammation, chronic (moderate severity).

Female dog UB 89. Face, abscess, inflammation, chronic (moderate severity).

Dose: 50.0 mg/kg/day. Male dog MH 95. Paw pad, right hind lesion, hyperkeratosis (severe), inflammation, chronic (moderate severity).

Female dog DH 99. Skin foot, right hind, lesion; inflammation chronic (moderate severity). Skin neck lesion, chronic inflammation of slight severity.

Female dog BX 99. Lesion of left and righ hind paw pads, hyperkeratosis (moderate severity), chronic inflammation (moderate severity).

Male dog CQ 99. Prepuce, raw sore. Epidermal inclusion cyst (slight severity).

The histological appearance was also characterized by the dermal infiltration of neutrophils and lymphoid cells.

SUMMARY

Males: A dose level of 2.0 mg/kg showed no apparent differences for effects from the control group.

A dose evel of 5.0 mg/kg showed no apparent difference for effects from the control group, with the exception of 2 dogs manifesting skin lesions. It is noted here that the number of skin lesions which were observed during the course of the experiment may not have been equal to those tabulated at histological evaluation. This is not unexpected since some lesions healed spontaneously prior to necropsy, some lesions were treated and were healed prior to necropsy and others were observed at necropsy and during histopathological examination.

A dose level of 15.0 mg/kg produced emesis and diarrhea which occurred at a greater frequency than either controls or the lower dose levels. Additionally, two animals manifested skin lesions. No other apparently meaningful effects were observed.

A dose of 50 mg/kg produced emesis and diarrhea which occurred at a greater frequency and quantity than the next lower dose level. One male which had received lactated Ringers solution early during the experiment was somewhat dehydrated but stable and responsive during the course of the experiment. Four males were observed as having skin lesions, one of which persisted to termination (Animal CQ 99, see also attached). Body weights were statistically significantly decreased from week 19 thru week 26. The mean corpuscular hemoglobin concentration was statistically significantly lower than Groups I, II and III at 180 days. Albumin was statistically significantly lower at 180 days. Calcium levels were statistically significantly lower at 120 days. The liver, kidney and adrenals were statistically significantly increased for organ to body weight ratio but only liver was statistically significantly increased for organ to brain weight ratio.

Females: A dose level of 2.0 mg/kg showed no apparent differences for effects from the control group with one exception. One female showed one lesion (open sore of the neck) at approximately 48 days post-dosing. The lesion was observed for 4 weeks but not treated. The animal was observed to scratch this lesion open repeatedly. The animal behavioral response to this lesion was similarly described for other animals at higher doses where a lesion was observed. This lesion apparently healed spontaneously. This lesion was previously reported during the review of the first 90 days of this study.

A dose level of 5.0 mg/kg showed no apparent differences for effects from the control group with one exception which was the manifestation of skin lesions in five animals--four animals manifested lesions during the in-life segment of the experiment and one manifested a lesion during histopathological examination.

A dose level of 15 mg/kg showed emesis and diarrhea occurring at a frequency and quantity greater than that of controls or the lower dose levels.

One female became severely depressed and dehydrated. The general health deteriorated with attendant weight loss, anorexia and the appearance of multiple abscesses (confirmed for staphylococus). The animal was euthanized on day 159 of the study. This was the only animal sacrificed inter-currently.

Three animals of this group manifested skin lesions. One of the animals was the one sacrificed inter-currently.

A dose level of 50 mg/kg manifested an emesis and a diarrhea to a greater extent than seen in controls or the next lower dose level. One female of this dose group remained 0-5% dehydrated for the entire period of the experiment. However, this animal was generally responsive during the course of the experiment and its general health appeared to be good. Four animals manifested skin lesions which were observed during the in-life portion of the experiment. Body weight was generally depressed during the course of the experiment. Total weight gain was also depressed at termination. The albumin level was statistically significantly decreased at 120 and 180 days and depressed at 150 days.

Males and Females Combined Values: Some effects only became apparent when the values for males and females were combined. These events occurred primarily at the high dose. The red blood cell (RBC) count was statistically significantly decreased at 180 days when male and female values were combined at the high dose. The RBC values when viewed separately by sex were generally depressed when compared to control values at 120, 150 and 180 days at the high dose. The hematocrit (HCT) for combined values at 180 days in the high dose group were statistically significantly lower compared to controls. The hematocrit values appeared to be generally depressed for separate sexes at 120, 150 and 180 days and the combined values for sexes at 120 and 150 days. Hemoglobin (Hg) values were shown to decrease with an increased dose for the separate values of sexes as well as the combined values for sexes in the high dose group at 180 days. A statistically significant

decrease was however only observed for the combined values of sexes at 180 days in the high dose group. Albumin values for the combined values of sexes were statistically significantly decreased at the high dose group at 120, 150 and 180 days. Calcium values for the combined values of sexes at 50 mg/kg were statistically significantly decreased at 120 and 180 days.

Discussion: Males and females mainifested emesis and diarrhea comparable to the control animals at doses of 2.0 and 5.0 mg/kg. Increased doses of compound led to a progressive increased emesis and diarrhea which were substantially above control values. It has been shown in previous dog studies that the emesis is mediated in large part, through the central nervous system. The compound is also a gastro-intestinal irritant. Dehydration, most likely a secondary effect of water and electrolyte loss was observed in the high dose group. The treatment with Ringers solution to replenish the electrolyte loss was effective in reversing the dehydration but was not totally corrective. Food consumption was generally comparable between treated and control groups. Body weights for females in the high dose group were generally depressed during the course of the study and were statistically significantly decreased for males from week 19 thru week 26. It would appear that the loss of weight is either secondary to the effects of emesis and/or diarrhea or a direct systemic effect of unknown definition resulting from a decreased food utilization. Whether or not the causative factor is direct or indirect it appears that the following results are inter-related events -decreased body weight, anemia, decreased albumin, decreased calcium, and increased liver weight. Serum calcium is protein bound primarily to albumin, and here in the absence of other evidence to the contrary, the decreased calcium level is probably a secondary effect to the decreased serum albumin although other probabilities can not be totally exlcuded. The serum albumin levels may be secondary to the anemia in that the regeneration of hemoglobin protein has priority over that of plasma protein. The decreased body weights and increased liver weight (histological examination of liver tissue showed that the tissue was normal) may also be reflective of protein metabolism irregularities or imbalance to some extent, although fluid loss and imbalance within the organism certainly can not be ignored. The extent to which the skin lesions contributed to the dehydration is not known but was probably minor by comparison to the diarrhea and emesis. It is also pointed out here that no evidence of hemo-concentration was evident thus further substantiating the case for anemia. Neurological findings appeared to be comparable between groups. Any differences which might exist could be attributed to electrolyte imbalance or a generalized depression. The examination of the spleen revealed

no differences from controls at 180 days contrary to what was observed at 90 days.

One female animal in the low dose group (2.0 mg/kg) manifested a skin lesion. It is this reviewer's opinion that this effect at this level is either dose related or compound related, with the effect occurring more often at the higher doses but in a randomly distributed manner. This position is taken in view of the number of lesions observed at the higher levels, the duration of the lesions, the animal behavioral response reported (i.e., observers' comments), location of the lesion and a seeming correlation between open sores of the neck and right hind paw which was observed in some animals.

These skin lesions are either a result of systemic toxicity or the result of direct topical exposure to the test article, leading to itching, scratching and subsequent formation of lesions. Topical exposure may be considered through animal contact of its own emesis and feces which would litter and contaminate the animal itself or the cage floor. Male dogs did not show lesions of the skin at a dose level of 2.0 mg/kg.

Conclusion: The dose of 2.0 mg/kg/day can not be considered, at this time, a definitive NOEL for this study and can not at this time be considered supportive of permanent tolerances, unless some rationale is presented that the skin lesions are the result of a non-systemic effect. If the question of the skin lesions is resolved, a no-observable-effect-level could not be established until the question of the spleen weight at 90 days is resolved.

If the question of the skin and spleen weight is resolved the NOEL for the six-month dog study could be established at 5.0 mg/kg/day.

Lowest Effect Level: 2.0 and 5.0 mg/kg/day based upon skin lesions; decreased spleen weight at 90 days at all dose levels (2.0 mg/kg/day assumed) both sexes.

No-observable-effect-level: less than 2.0 mg/kg/day (see conclusion).

Classification: Core-Guideline.

FLUVALINATE

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Subject: Six Month Subchronic Dog Study 90-day Interim Kill Report (90-Day Dog Study).

Test Compound: ZR-3210 Technical (Racemic Mixture), Fluvalinate, Mavrik(R)

Accession No: 070097

Testing Facility: Elars Bioresearch Laboratories, Inc., Fort Collins, Colorado 80524.

Project No: 1503

Responsible Professionals:

Jeanne de Ward - Study Coordinator

L. Steven Beck - Study Director

Donald N. Kitchen - Consulting Pathologist

Douglas I. Hepler - V. P. Toxicity Evaluation Division

Testing Period: December 21, 1979 - April 13, 1980

Report Submitted to Sponsor: June 1980

Purity of Test Material: 93.8% (first shipment); 93.0% (second shipment)

Batch or Lot No: Analysis No. 0979-069 Run 7 (first shipment); Analysis No. 0280092 (second shipment).

Stability: Stable for at least 12 months when stored in sealed glass containers and exposed to artificial light at 25 and 42°C. Stable in preparation and under the conditions of the study.

Materials and Methods: Test Material: (Note: The materials and methods section has been written to accomodate both the 90-day and 180-day dog study as one was the continuation of the other.) Preparation of the stock solutions was conducted on a weight/weight basis (mg active ingredient/grams of total solution). Corn oil was used as the vehicle (Westman Commission Company). Two stock solutions, 30 mg. a.i./ml total solution and 300 mg. a.i./ml total solution, were prepared and used in appropriate doses to fill gelatin capsules for oral administration. The 300 mg. a.i./ml solution was used for capsule preparation at the two highest dosage levels; the 30 mg. a.i./ml solution was used for the remaining dosage levels. Capsules were stored at room temperature and were protected from cross-contamination and excess moisture.

Stock solutions were prepared every 28 days and examined visually for homogeneity. Solutions were stored in amber glass bottles at room temperature until fresh solutions were prepared and were restirred prior to capsule preparation. Two milliliter samples of freshly prepared solutions were assayed by Elars' Chemistry Department to ensure proper formulation. Assays of both stock solutions were within 10% of the specified concentrations.

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Replicate samples of the stock solution were sent to the sponsor for confirmatory analysis. Additionally, two sample capsules for each dosage level were assayed every two weeks to ensure proper concentration. Results of these assays showed all capsules to be within 10% of the required doses.

Test System: Twenty (20) purebred Beagle dogs, evenly divided as to sex, were received from Daisy Hill, Inc., Denver, Colorado. Eighty (80) more purebred Beagle dogs, evenly divided by sex, were received from Laboratory Research Enterprises, Kalamazoo, Michigan. Each dog was uniquely marked and individually housed in standard stainless steel cages. Animal quarters were environmentally controlled for temperature and humidity.

Dogs were offered Purina Laboratory Dog Chow(R) ad libitum daily at least one hour prior to dosing. Dogs were fasted, however, for at least 12 hours prior to collecting blood samples. Drinking water was provided ad libitum throughout the study and was checked at least twice daily. The water source, from a municipal tap, was analyzed during the winter months and quarterly thereafter.

Study Design: Dogs were acclimated to laboratory conditions for 3 weeks prior to dosing, and were approximately 5-6 months of age at initiation of dosing. It is noted here that the pilot study indicated that older, heavier dogs exhibited less severe emesis than younger dogs and would thus help to achieve better quantified systemic dose levels. During this period, all dogs were treated for intestinal parasites and immunized against distemper, hepatitis and leptospirosis. Two weeks prior to dosing, each dog was given a complete physical examination which included opthalmic and fecal exams, urinalysis, hematology and clinical chemistry. Body weights and food consumption for each dog were recorded for three weeks prior to the initiation of the study.

Ninety-two healthy dogs (46 males and 46 females) were randomly assigned to five treatment groups: Group I (vehicle control), Group II (2 mg/kg), Group III (5 mg/kg), Group IV (15 mg/kg) and Group V (50 mg/kg). Since dogs were obtained from two suppliers, dogs were first classified according to the supplier, separated by sex and ranked by weight within each sex. Using a table of random numbers, dogs were then assigned to test groups. Dogs were further divided into four equivalent replicates, within treatment groups, in order to limit the number of dogs introduced to the test material each day. Random assignment to Replicates A, B, C, and D was achieved by using a table of random numbers. Please refer to the table immediately below:

Replicate Assignment and Initiation to Test Material

Date of Initiation	Replicate	Sex	Number of Dogs Group I	Group II	Group III	Group IV	Group V
1/11/80	A	M	2	1	3	2	3
		F	3	2	2	3	2
1/12/80	B	M	3	2	2	3*	2
		F	2	1	3	2*	3
1/13/80	C	M	2	1	3	2	3
		F	3	2	2	3	2
1/14/80	D	M	3	2	2	3	2
		F	2	1	3	2	3
Total			20	12	20	20	20
Interim Kill (90 days)			4 M/F	None	4 M/F	4 M/F	4 M/F
Continued on Study Dogs			12	12	12	12	12

* One female dog was inadvertently recorded in the study notebook as a male. This error was discovered at the 180-day mark. All Group IV means were recalculated to reflect this error.

Dogs in Groups II, III, IV and V were introduced to the test material in gradual increments for 10 days prior to day 1 of dosing, at which time each received the full dosages according to weight and treatment group. These incremental doses were given in an effort to reduce the frequency of emesis since the pilot study gave some indication that the dogs accomodated to the test material with time.

Dogs were administered full dosage of the test material as indicated in the above table. Control groups received sham-dosed capsules containing 1.0 ml of corn oil vehicle during the period of incremental dosing and throughout the 90 consecutive days of dosing. Capsules were prepared twice weekly for each replicate and doses were adjusted weekly according to body weights.

Blood and urine were collected from individual dogs throughout the 90-day (180 day) study according to their replicate assignments. Likewise, necropsy, performed at 90 days of dosing for the 32 randomly selected dogs, was scheduled according to replicate assignments.

Four dogs of each sex from the control group, 5, 15 and 50 mg/kg group were sacrificed and necropsied after 90 days of dosing. The animals to be sacrificed had previously been selected by means of a random number table. Terminal body weights were obtained on all animals prior to necropsy. No animals

from the low dose group (Group II, 2.0 mg/kg) were sacrificed at the 90-day period.

The remaining 60 animals continued to be dosed, observed and tested for all study parameters until study termination at six months.

Observations and Measurements: Dogs were observed daily for general appearance, behavior elimination, and signs of toxic or pharmacologic effects. Daily observations were recorded. The frequency of emesis and diarrhea and a description of lesions were recorded. Each dog was weighed weekly. Food consumption was measured daily. Daily mean food consumption was determined for each dog by dividing the total feed consumed during the week by seven (7) days.

Opthalmic examinations were also performed on all dogs at 90 and 180 days. Opthalmoscopic exams were conducted in accordance with Canine Opthalmology by William G. Magrane or any other recognized and accepted text on opthalmology. Eyes were dilated with 1% Mydriacyl prior to examination. Eyes were examined externally and internally. A Schirmer tear test was also conducted. (Detailed procedures may be found in Accession No. 070098.)

A neurologic exam was also performed on all dogs of groups, I, III and IV prior to sacrifice. The examination entailed the following:

Cranial Nerve [CN] Examination

pupil size and reflex	-	C.N.	2 & 3
menace reflex	-	C.N.	2 & 7
sensory and motor to lips	-	C.N.	5 & 7
palpebral reflex	-	C.N.	5 & 7
swallowing reflex	-	C.N.	9 & 10
observe for head tilt	-	C.N.	8
observe for strabismus or abnormal eye placement	-	C.N.	3 & 4 & 6 & 8

Spinal Cord Exam

proprioception
motor system

Sensory Examination

pain perception

The neurological findings, carried out on a blind basis, were classified as either normal, equivocal or positive with description. A more detailed description may be found in accession 070098.

Blood was drawn from each dog two weeks and one week before dosing and afterwards at 30-day intervals until termination of testing and necropsy. Individual hematology and clinical chemistry determinations were recorded and mean group determinations calculated. Hematology parameters included: RBC, HCT, Hb, platelets, MCH, MCHC, APTT (activated partial thromboplastin time),

prothrombin time, WBC, segmented neutrophils, band neutrophils, lymphocytes, monocytes, eosinophils and basophils. Methemoglobin at day 150 only was also measured. Clinical chemistry parameters included: total protein, albumin, globulin, A/G Ratio, AP, SGOT, SGPT, LDH, glucose, BUN, total bilirubin, cholesterol, RBC cholinesterase, serum cholinesterase. Calcium, potassium and sodium were determined at 180 days only. RBC and serum cholinesterase assays were performed on all dogs at the 60, 90 and 180-day bleeding. Brain cholinesterase assays were conducted on each of the dogs killed at the interim sacrifice, and at the final sacrifice.

Urine was collected from each dog two weeks and one week prior to dosing and afterwards at 30-day intervals until termination. Urinalysis parameters included: protein and glucose concentration, specific gravity, ph, ketones, bilirubin, urobilinogen and a microscopic elements examination.

Immediately prior to termination animals were fasted overnight, weighed the next morning, anesthetized with sodium pentobarbital, i.v., and exsanguinated by cardiac puncture. A gross necropsy was conducted and the following organs were extirpated and weighed; liver, kidneys, heart, brain, spleen, gonads, adrenal glands, thyroid glands (with the parathyroids) and pituitary gland. Organ/body weight ratios and organ/brain weight ratios were calculated.

Selected tissues were removed from each animal and fixed in 10% buffered formalin solution (eyes and testes in Bouin's solution) for histological examination. Tissues were prepared following standard histological practices. Tissues were sectioned at 4-5 micron thickness and stained with hematoxylin and eosin.

The following tissues and organs were subjected to histopathological examination:

- Adrenal gland
- Aorta
- Bone marrow
- Brain
  - Cerebrum
  - Cerebellum
  - Pons
- Cecum
- Colon
- Esophagus
- Eyes with optic nerve
- Gall bladder
- Heart
- Kidney
- Liver
- Lung
- Lymph node
  - Cervical
  - Mesenteric
- Mammary Gland
- Muscle
- Ovaries
- Pancreas
- Parathyroid
- Peripheral nerve (sciatic)
- Pituitary gland
- Prostate
- Salivary gland (submaxillary)
- Skeletal muscle (capsule & sarcolemma)
- Skin
- Small intestine
  - Duodenum
  - Jejunum
  - Ileum
- Spinal cord (3 levels)
- Spleen
- Stomach
  - Cardia
  - Fundus
  - Pylorus
- Testes with epididymides
- Thymus
- Thyroid gland (with parathyroid)
- Trachea
- Urinary bladder
- Uterus (corpus & cervix)

Any other tissue or organ showing gross lesions or alterations was also evaluated microscopically.

Statistical Analysis: An analysis of variance test was performed on each listed measurable parameter. Whenever a probability value of less than 0.05 was found, a Tukey's HSD procedure was performed in order to detect statistically significant differences among groups. Data which were analyzed statistically were body weights, feed consumption, hematology, clinical chemistry, absolute organ weights and organ/body weight and organ/brain weight ratios.

Clinical Observations: The most frequently detected dosage-related sign was emesis which occurred frequently in all dogs and almost daily in some dogs receiving 50 mg/kg. Accomodation to the test article, in terms of frequency of emesis was not observed. Emesis occurred as frequently after 90 days of dosing as it had at the beginning. The frequency and quantity of emesis in those animals receiving 15 mg/kg were generally 3 to 4 times less then that observed in the next highest dose (50 mg/kg). Emesis occurred infrequently in the vehicle control group as well as those animals receiving 2 and 5 mg/kg/day. No detectable differences in either the frequency or quantity of emesis was noted among these latter groups.

The incidence of diarrhea observed in this 90-day study followed a pattern similar to that of emesis with the diarrhea being most frequent in the high-dose group and occurring on the average of 2 to 3 times less often at 15 mg/kg than at 50 mg/kg. The incidence of diarrhea was comparable between controls and the two low-dose administered groups.

Dehydration occurred in several high-dose (50 mg/kg) dogs at various times, but appeared consistently in four females and one male. In each of these dogs, diarrhea and vomiting occurred almost daily; however, this was also reportedly true of other animals in this high-dose group who never became dehydrated or who were dehydrated only sporadically. The four female dogs also appeared emaciated and debilitated and all five dogs had extremely oily and unkempt appearing hair coats and seemed lethargic and depressed. One female appeared to improve spontaneously in the latter part of the third month of the study, gaining weight and becoming more alert and active. Two other females, however, remained stable, usually approximately 0-5% dehydrated but still somewhat responsive. One female and one male became so severely dehydrated and debilitated that they were placed on fluid therapy. These dogs were consistently 8-10% dehydrated, severely depressed and debilitated, and both showed an overall weight loss from the beginning of the study. Lactated Ringer's Solution (Na-lactate, NaCl, KCl and $CaCl_2$) was administered subcutaneously for one week, after which both dogs were weaned gradually from the fluid regimen. Both dogs responded immediately to the therapy, becoming more alert and active with lessening dehydration, although it never completely disappeared. The improvement continued after the fluids were withdrawn and both dogs were in relatively good condition at the 90-day interim period. Of the 5 severely affected dogs, 3 were interim kill dogs (all female) and the remaining two (1 male and 1 female) were followed through for the next 90 days.

Localized lesions persisting for weeks were reported in all groups receiving the test article. Although a log-dose response was not generally evident, a compound-related effect appeared to be present (see attached).

One lesion (open sore/neck) was observed in the low-dose group with 8, 3 and 6 lesions occurring at the next successive higher levels. Generally, the rate of lesion occurrence was one per dog. The described lesions were generally categorized as either an open sore or an irritation. Open sores were generally in the neck area or the right hand paw. Irritation was generally noted in the area of the prepuce or the vulva. The number of lesions appeared to be nearly equally distributed between sexes. However, females did hold a slight edge over males with the data indicating that the females apparently suffered the greater severity (neck vs. hind paw; see attached). No lesions were recorded for controls.

It was reported that dogs with lesions appeared to suffer from pruritus; they licked, chewed or scratched the affected areas repeatedly. The pruritus appeared to be more severe shortly after dosing, since dogs which were quiescent in the morning chewed and scratched themselves two to three hours after they were dosed.

Numerous treatments were attempted. The affected areas were wrapped or bandaged, Elizabethan collars were placed on the dogs and finally, if necessary, various topical medications were applied. The application of various medications (topical antibiotics) was reserved only for the most refractory cases. Staphyloccocus aureus was identified in four dogs and beta-hemolytic streptococcus in two dogs.

Body Weights: Mean body weights for males reveal no statistically significant differences between treated and control dogs at any measurement time. The mean body weights for females were, however, statistically significantly lower ($p < 0.05$) than female controls at 12 and 13 weeks at the high-dose level only. The combined male and female body weights were, however, statistically significantly lower ($p < 0.05$) than combined control weights at weeks 12 and 13 at the high-dose level.

Graphical representation of male body weight data indicated that all groups with the possible exception of Group II remained below control values at all time intervals. Graphical representation of female body weight data revealed an almost immediate divergence from controls to a lower level for those animals in the high-dose group. From initial to final readings, the curve was essentially flat for female body weight in the high-dose group.

Weekly body weight gain was statistically significantly decreased for males in the high-dose group at weeks 1, 3, and 4 when compared to controls. The decreased weight gain for males resulted in a combined weight decrease for males and females for the same four-week interval. Female values were comparable to controls for this 4-week period. The total weight gain was statistically significantly less for males, females and their combined weights at 13 weeks for the high-dose group only.

Food Consumption: Food consumption was not statistically significantly different from control values for either sex when considered separately or in combination. However, feed consumption was noticeably depressed at the high-dose levels.

Opthalmic Examinations: No abnormalities or changes were observed in the Schirmer tear test. Superficial and adnexal examinations at 90 days revealed an occasional corneal irregularity or distichia, but these were not believed to be treatment-related. Funduscopic observations also revealed lesions. These lesions were, however, either present at the pre-test examination or fairly uniformly distributed between all groups at the 90-day examination.

Neurologic Examination: Results from the neurologic examinations performed at 90 days on all dogs from the control group, the 5 mg/kg group and the 50 mg/kg group were as follows: two abnormalities were observed in controls, three at the 5 mg/kg dose level, and seven at the high dose. Hind limbs were involved more frequently than the front limbs and the patellar reflex was affected more frequently than the other parameters tested. The patellar reflex abnormalities seen in the high-dose group were either hyperreflexia (1 male and 1 female) or hyporeflexia (2 females). Three of the dogs had both right and left patellar involvement.

Clinical Pathology: Hematology: The Red Blood Cell Count (RBC) was statistically significantly lower for females only in the high-dose group and only at the 90-day reading. This result at this time period resulted in a statistically significant decreased RBC count for the combined values of both sexes at the 90-day interval. Males showed no statistically significant decreases at any time. However, the RBC count for males was depressed at 90 days in the high-dose group. Hematocrit (HCT) was statistically significantly lower for males and females at the 90-day reading in the high-dose group. Female hemoglobin values were statistically significantly decreased in the high-dose group at 90 days. This depression was also reflected in the combined values for males and females at the 90-day interval in the high-dose group. Males showed no statistically significant decreases in hemoglobin values at any period. However, the hemoglobin value at the 90-day reading in the high-dose group of males was noticeably depressed. Platelet count was statistically significantly elevated in males at 90 days in the high-dose group. Values for females in the high-dose group at 90 days were elevated but not significantly elevated. Values were statistically significantly increased for the combined sexes in the high-dose group at 60 and 90 days. Values for males and females when considered separately were higher in the high-dose group at 60 days but were not statistically significant. No statistically significant changes were noted for values of mean corpuscular hemoglobin. Values for mean corpuscular hemoglobin concentration were elevated. Activated Partial Thromboplastin Time (APTT) and Pro-time (prothrombin time) values were comparable to control values. White blood cell count and WBC differential count showed no statistically significant changes at any dose level for any period of time when compared to controls. Values for total protein for the combined sexes and for the sexes considered separately were generally comparable to the control readings at most time intervals and doses. Total protein values were statististically significantly decreased for males at the 30- and 90-day readings for groups II and III, respectively. A statistically significant decrease was recorded for combined values of males and females only at 90 days in group III (5.0 mg/kg). Values for females were comparable to controls at all doses and time intervals. Males showed a statistically significantly decrease in albumin values only at 90 days for the group receiving 5.0 mg/kg. Females manifested a statistically significant decrease only at 90 days in the high-dose group of 50 mg/kg. Values for the combined sexes showed statistically significant decreases in albumin values at the same dose levels and time periods as recorded for the separate sexes. Globulin values were not statistically significantly different from control values. The albumin/globulin ratios (A/G ratios) were not statistically significantly different at any period. Values for glucose can generally be considered as not significantly different from control values. BUN was comparable for all groups at all time intervals. Total bilirubin was comparable for all groups at all time intervals. Alkaline phosphatase was also comparable for all groups at all time intervals. Values for calcium were statistically significantly lower for the combined values of sexes at 50 mg/kg and 60 and 90 days. Values for calcium, when considered separately by sex, were not significantly different from controls at any period. Values for Na and K can be considered as comparable to controls at all periods. SGOT and SGPT values for females were comprable at all periods. SGOT values for males were

statistically significantly lower at 30 and 60 days in the high-dose group. Values for combined sexes were statistically significantly lower for 30 and 60 days at the high-dose level. SGPT levels for males were statistically significantly lower only at 30 days in the high-dose group. A statistically significant decrease in SGPT levels was also recorded at 30 days in the high-dose group for the combined values of the sexes. Cholesterol values were comparable for treated groups when compared to controls at all time periods. LDH values were comparable between treated groups and control groups. RBC cholinesterase values were statistically significantly lower for males, females and their combined values at 90 days. Serum and brain cholinesterase values were comparable at all doses and time intervals. Values for specific gravity (urine) and ph (urine) were presented for combined sexes and were not statistically significantly different from the control group. No statistically significant differences were noted among groups for the other parameters listed at 30, 60 or 90 days for urinalysis. Absolute values for organ weights and organ weight ratios (either body or brain weight) showed spleen to be consistently decreased for all three types of measurement at the high-dose. Decreased spleen weights were statistically significantly lower for both sexes and their combined values at 50 mg/kg. A progressive decreased spleen size was observed with increased dose. Statistically significant changes were observed for other organs in the high-dose female group for organ weight to body weight ratios. However, these differences disappeared when organ weight to brain weight ratios were compared. Therefore, with the exception of the spleen, all other organ ratios appeared to be not biologically meaningful.

No treatment-related abnormalities were observed at gross necropsy.

Histopathology did not reveal any compound-related effects for the tissues examined. It was reported that spleen and skin were not remarkable.

Animals with Lesions Compared to Those Animals Showing Dehydration Compared to those Showing Neurological Changes. A side-by-side comparison of animal numbers was made to establish whether or not an apparent correlation existed between these three parameters and the animals in which they appeared. It was concluded that no correlation appeared evident.

Spleen - Male and Female: Absolute Organ Weight/Body Weight and Brain Weight Ratios. A tabular representation of the indices noted are presented below:

MALES

Dose (mg/kg)	Absolute Wt. (g)	B. Wt. Ratios	Br. Wt. Ratios
0	35 ± 8	0.34	47 ± 11
5	28 ± 4	0.31	37 ± 5
15	26 ± 4	0.26	34 ± 4
50	*18 ± 5	*0.20	*22 ± 5

*($p < 0.05$)

Note: Animals from Group II (2.0 mg/kg) were not sacrificed at the interim kill.

FEMALES

Dose (mg/kg)	Absolute Wt. (g)	B. Wt. Ratios	Br. Wt. Ratios
0	34 ± 6	0.41	45 ± 6
5	26 ± 8	0.32	36 ± 11
15	24 ± 3	0.29	30 ± 3
50	*16 ± 2	*0.24	*22 ± 3

*($p < 0.05$)

Summmary - Males

Dose: 2.0 mg/kg: The frequency and quantity of emesis and diarrhea was shown to be comparable to controls. Total protein was decreased at the 30 day reading but the data for this parameter showed no changes with respect to time or dose.

Dose: 5.0 mg/kg: The frequency and quantity of emesis and diarrhea was shown to be comparable to controls. The results of the neurological examination at 90 days also appeared to be comparable for this group to control animals. Total protein and albumin were statistically significantly decreased at 90 days. However, these decreases were not noted at the next two higher doses. Additionally, no correlation appears readily evident between the open sores observed (two males) and the decreased total protein and albumin levels at this time period.

Dose: 15 mg/kg: Emesis and diarrhea were greater than controls at all time periods, but also much less than the next higher dose level. Only one male revealed an open sore of the right hind paw. No other apparently meaningful effects were observed.

Dose: 50 mg/kg: Emesis and diarrhea were greatly increased in frequency and quantity over controls at this dose level. Dehydration was transient in some animals (sex not specified but probably included some males) and constant and severe in at least one male. This animal was treated with Ringers lactate solution and was reportedly in relatively good shape at the 90 day period. Mean body weights and food consumption were depressed, but not statistically significantly lower than controls at anytime. Body weight gain was statistically significantly lower than controls only at the 90 day reading. Two lesions were observed in two animals, one in the neck and one with an irritated prepuce. The size and depth of the neck lesion was not reported for this dog or any other dog, male or female, at any time period. Findings for the neurological examination appeared to be equivocal for males when compared to controls. Hematocrit was statistically significantly lower at 90 days accompanied by a depressed hemoglobin and RBC count. Platelet count was elevated at 60 days and significantly increased at 90 days. RBC cholinesterase was statistically significantly decreased. SGOT and SGPT values were decreased at 60 and 90 days, but apparently were not biologically meaningful.

Pathology and Organ Weight, All Groups: No compound-related gross or microscopic pathology was reported. Organ to body weight and brain weight ratios for spleen appeared to reveal a dose response decrease with increased dose. This was the only consistent finding for organ weights.

Comparative Inspection of Male Animals Manifesting Lesion vs. Dehydration vs. Neurological Changes. No correlation appeared evident between the parameters and the animals in which they were observed.

Summary - Females

Dose: 2.0 mg/kg: The frequency and quantity of emesis and diarrhea was shown to be comparable to controls for all time periods. One female showed one lesion (open sore of the neck) approximately 48 days post dosing. The sore was observed for 4 weeks but not treated. The animal was observed to scratch this lesion open repeatedly. The animal behavioral response to this lesion was similarly described for other animals at higher doses where a lesion was observed. This lesion apparently healed spontaneously.

Dose: 5.0 mg/kg: The frequency and quantity of emesis and diarrhea was shown to be comparable to controls for all time periods. Five females were described as having lesions -- two with open sores of the neck, one at the tip of the tail, and two with open sores of the right hind paw. The animals chewed and scratched at these lesions repeatedly.

Dose: 15 mg/kg: The frequency and quantity of emesis and diarrhea were substantially greater than control values, but markedly less than the next higher dose level. Only two animals were shown to have lesions -- one with an open sore of the neck and one with interdigital pyoderma of the left front paw.

Dose: 50 mg/kg: The frequency and quantity of emesis and diarrhea was substantially greater than in any other group. Four females manifested lesions at this dose -- one had an open sore of the neck, the other of the thigh. The other lesions (irritation) were generally in the area of the genitals. Four females also showed a constant and in some cases a very severe dehydration. One animal was treated with Ringers Lactate Solution. Body weight and body weight gain were statistically significantly decreased at the 90 day reading. Food consumption was generally depressed. Neurological examination reported one female with hyper- and 2 females with hypo-reflexia of the patellar tendon. RBC, Hb and HCT readings were statistically significantly lower at 90 days. Platelet count was raised at 60 days and statistically significant at 90 days. RBC cholinesterase was decreased significantly at 90 days. Albumin values alone were statistically significantly decreased at 90 days.

Pathology and Organ Weight All Groups: No compound related gross or microscopic pathology was reported. Organ to body weight and brain weight ratios for spleen appeared to reveal a dose response decrease with increased dose. This was the only consistent finding for organ weights.

Comparative Inspection of Female Animals Manifesting Lesions vs. Dehydration vs. Neurological Changes. No correlation appeared evident between the parameters and the animals in which they were observed.

Discussion: Males and females manifested emesis and diarrhea comparable to the control animals at doses of 2.0 and 5.0 mg/kg. Increased doses of compound led to a progressive increased emesis and diarrhea which were substantially above control values. Dehydration, most likely a secondary effect of water and electrolyte loss, was observed in the high dose group. The treatment of severely dehydrated animals with Ringers Lactate Solution to replenish electrolyte loss, was effective in reversing the dehydration, but was not totally corrective. The decreased food consumption, body weights and body weight gains at the high dose level were not surprising in light of the above described effects at the high dose level.

The neurological findings in females of the high dose group appear to be secondary effects in light of the reportedly generally depressed state of the four females examined. No great neurological differences were observed between males of the examined groups. It would therefore appear that no adverse neurological effects directly attributable to compound were noted for the parameters tested.

Hematocrit (HCT), hemoglobin (Hb) and red blood cell count (RBC) were decreased and suggest an anemia. The decreased hematocrit may also be responsible for the decreased RBC cholinesterase values. The increased platelet count may be reflective of petechial hemorrhaging in the gastrointestinal tract observed in the pilot dog study but not this study. No blood was observed in the urinalysis and none apparently in the feces. A decreased HCT, Hb, and RBC count with an increased reticulocyte value was seen in the 90-day rat study. This reviewer believes the anemia [redacted] real and not totally a secondary effect of internal bleeding which may or may not exist.

may be (AB1C)

Gross and histopathology revealed no compound related effects. It is important to note here that no animals sacrificed at the interim kill were observed having skin lesions at the period of the interim kill, although those animals continued on for the next 90 days did show lesions. All those animals sacrificed at the interim period were previously preselected in a random fashion for the interim sacrifice and were cured prior to sacrifice. Those animals that were not previously pre-selected for the interim kill had lesions at 90 days and were continued on for the remainder of the experiment.

Spleen organ-to-body weight and organ-to-brain weight ratios showed apparent dose-related effects at dose levels of 5, 15 and 50 mg/kg, in those animals sacrificed at the period of the interim kill. The effect at the high dose level was statistically significant. It is pointed out here, however, that gross and histopathology were not remarkable for this organ. We offer two possible explanations for the observations recorded. Since the gross and histopathology were not remarkable but the spleen weight was decreased with increasing dose, a possible explanation would be that the observed effects of decreased spleen weight are secondary to the physiological act of vomiting and compression of the viscrea resulting in a proportionally decreased "blood reservoir" capacity. A physical compression of the spleen would decrease the weight of the spleen simply because less blood volume was contained within it. A second possible explanation involves the method of sacrifice, exsanguination, and time to death. The physiological process of dying during the process of exsanguination may have by chance alone (or methodology) resulted in a dose responsive decrease in spleen weight, as determined by the amount of blood retained in the spleen at death.

This reviewer is not oblivious do the fact that no animals in the low dose group (2.0 mg/kg) were sacrificed at the 90-day interval, thereby precluding spleen weight data at this dose level. However, we would suggest that the organ-weight ratio at this level, if a true-dose-response-direct-effect does indeed exist, would be less than the effect at the 5.0 mg/kg level and may be no different by comparison to controls. Since the possibility exists that the dose of 2.0 mg/kg may be dose responsive to the effect on spleen, we would suggest that the dose-effect relationship is in the area of the "threshhold area for effect" and certainly not statistically significant.

One female animal in the low dose group (2.0 mg/kg) manifested a skin lesion. It is this reviewer's opinion that this effect at this level is either dose related or compound related, with the effect occuring more often at the higher doses but in a randomly distributed manner. This position is taken in view of the number of lesions oberved at the higher levels, the duration of the lesions, the animal behavioral response reported (i.e., observers' comments), location of the lesion and a seeming correlation between open sores of the neck and right hind paw which was observed in some animals.

These skin lesions are either a result of systemic toxicity or the result of direct topical exposure to the test article, leading to itching, scratching and subsequent formation of lesions. Topical exposure may be considered through animal contact of its own emesis and feces which would litter and

contaminate the animal itself or the cage floor. If the lesions are log-dose responsive and of a systemic nature, then one could say that the low dose effect observed may be in the "threshold area of response" as based on the number of females showing this effect at this level, which was one female, the duration of the lesion and the reversibility of the lesion in the absence of medical treatment. We also take the opportunity to point out here that male dogs did not show lesions of the skin at this dose level. If the skin lesion is a result of topical exposure, then the avoidance of contact would be indicated.

Conclusion: (see also Comment paragraph written below). The dose of 2.0 mg/kg may be considered to be at or near the NOEL (i.e., "threshold level" for a no-observable-effect-level) as based upon the results and interpretation of the 90-day experiment. The primary considerations are the skin lesion in the low dose female as well as a possible (but not necessarily probable) effect on the spleen in male and female dogs.

Comment: The above review has addressed the scientific aspect of the study and has concluded that the lowest dose test is probably at or near the no-observable-effect-level for this coupound.

This study has been submitted in support of an experimental use permit on cottonseed with an attendant request for the establishment of temporary tolerances on this commodity.

In the consideration and establishment of temporary tolerances from a NOEL of a 90-day study, a safety factor of 2000 is applied. This reviewer has already indicated that in his opinion the effect manifested for spleen weight and skin lesion are in the threshold area of the dose response (i.e., the threshold level being that dose level which barely manifests a positive response or, to say it another way, just barely crosses over the line from a no-observable-effect).

This study is in support of an experimental use permit with tolerances. We suggest that when these effects are viewed from a regulatory viewpoint an ample margin of safety exists when residues of this pesticide remain on a raw agricultural commodity and are ingested orally.

We also point out here that, since the possibility exists that the skin lesions occur as a result of topical exposure, the avoidance of contact should be indicated with the appropriate precuationary labeling.

We also point out here that in any future petition for permanent tolerances the question of these lesions needs to be resolved prior to the issuance of permanent tolerances.

We would also like from the company some rationale as to the presence or absence of a pharmacological or toxicological effect on the spleen with regard to the decreased weights and weight ratios observed. We feel that this question is important since no animals were sacrificed from the low dose group

(2.0 mg/kg/day) at the 90-day interim kill. This reviewer is aware that no differences in spleen weights or ratios were reported at the six month period; none the less the question is raised and needs to be addressed.

Provisional LEL as based upon the following:

skin lesions; at 2.0 and 5.0 mg/kg/day.

spleen; decreased spleen weight at all dose levels (2.0 mg/kg/day assumed) both sexes.

Provisional NOEL; equal to or less than 2.0 mg/kg/day.

If the question of the skin lesions is resolved, a no-observable-effect-level could not be established until the question of the spleen weight is resolved.

If the question of the skin and spleen is resolved, the NOEL for the 90-day dog study could be established at 5.0 mg/kg/day. However, we note here that 5.0 mg/kg/day for the dog would be higher than the current provisional NOEL for the 90-day rat study. The NOEL for the 90-day study in rats would be 3.0 mg/kg/day if the question of the skin lesions is resolved.

Classification: Core - Minimum

Fluvalinate

Page_____ is not included in this copy.

Pages 33 through 55 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

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_____ The product confidential statement of formula.

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